

## Description:

One-Fusion DNA Polymerase is a unique artificial enzyme created on the basis of intellectual protein design planning by genetic engineering technique. The enzyme possess high fidelity feature. The processivity of the enzyme is very high, so the combination of processivity with fidelity results in dramatically increased yield of PCR products, very high sensitivity of PCR tests, ability to amplify "difficult" templates.

This dramatic increase in processivity results not only in shorter extension times, but also in more robust amplification and the ability to amplify long templates: really fast.

One-Fusion DNA Polymerase possesses the 5'->3' DNA polymerase activity, 3'->5' exonuclease activity and temperature-dependent strand-displacement activity and generates blunt ends in the amplification products.

## Applications:

- High Fidelity (Hi-Fi) PCR
- Cloning
- "Hi-Fi" - LD PCR
- "anticontaminated" PCR
- "direct blood" PCR

## Features:

- Superior fidelity – about 50x improvement compared to Taq Polymerase
- Excellent performance across a wide range of "difficult" templates
- Long range amplification of complex targets - > 10 kb from genomic DNA
- High speed PCR - reduce reaction times
- dUTP poisoning resistance
- Resistance to blood containing DNA samples (up to 20 % of blood)

**Unit definition:** One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP's into acid-insoluble form in 30 minutes at 75°C under assay conditions: 25 mM TAPS-HCl, pH 9.0 (at 25°C), 100 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1 mMβ-mercaptoethanol, 200 μM each dNTP, and 10 μg activated calf thymus DNA in 50 μl.

**Associated Activities:** Endonuclease and exonuclease activities were not detectible after 2 and 1 hours incubation, respectively, of 1 μg lambda DNA and 0.22 μg of EcoR I digested lambda DNA, respectively, at 72°C in the presence of 15-20 units of One-Fusion DNA polymerase.

**Storage buffer:** 20mM Tris-HCl, pH 8.0, 100mM KCl, 0.1mM EDTA, 1mM DTT, 50% Glycerol, 0.5% Tween 20, stabilizers

## Reaction buffers provided:

The product is supplied with 2,5x Reactionbuffer containing 3,75 mM MgCl<sub>2</sub>

**Storage:** store at - 20°C

## General protocol:

The optimal reaction conditions for One-Fusion DNA Polymerase may differ from PCR protocols for standard (Taq-like) DNA polymerases.

PCR conditions for one-Fusion DNA Polymerase is more similar in PCR conditions to "Phusion-like" DNA polymerases, e.g. the enzyme works better at elevated denaturation and annealing temperatures.

PCR reactions should be set up on ice. Prepare a master mix for the appropriate number of samples to be amplified.

**Note! It is critical that the One-Fusion DNA polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3'->5' exonuclease activity that can degrade primers in the absence of dNTPs.**

Component	50µl reactions	25µl reactions	Final concentration
PCR grade Water	Up to 50 µl	Up to 25 µl	
2.5x One-Fusion Buffer	20 µl	10 µl	1X
10 mM MIX dNTPs	1 µl	0.5 µl	0.2 mM each
Primers			0.3-0.5 mM each
Template DNA	optionally	optionally	
One-Fusion polymerase (2 U/µl)*	1 µl	0.5 µl	0.02 U/µL

\* results in 1.5mM Mg<sup>2+</sup>, as the final concentration.

In some cases we recommend to optimize Mg concentration in the range 1.5-2.5mM'.

We recommend to use 50µl reaction for the PCR with One-Fusion polymerase.

## Cycling:

Cycle Step	2-step amplification		3-step amplification		Cycles
	T°C	Time	T°C	Time	
<b>Initial Denaturation</b>	98°C	2-5 min	98°C	2-5 min	1
<b>Denaturation</b>	98°C	5-10 S	98°C	5-10 S	
<b>Annealing</b>	-		55-72*	10-30 S	25-35
<b>Extension</b>	72°C	15-30 S/Kb	72°C	15-30 S/Kb **	
<b>Final Extension</b>	72°C	1-3 min	72°C	1-3 min	1
	4°C	hold	4°C	hold	

\*Optimal T<sub>m</sub> for the primer pair recommended as T<sub>m</sub> of the lower primer, for the standard-oligos <20nt.

For One-Fusion polymerase T<sub>m</sub> of the primers should be corrected, as +3-5°C, comparing with Taq-based PCR conditions.

\*\*For non-complex DNA templates (plasmid DNA, phage DNA, BAC clone) extension time could be reduced up to 15 sec/Kb.

For complex DNA templates (human DNA) strongly recommended to apply extension time as 30 sec/Kb

## Order Information

Prod. No.	Description	Quantity
S9450	One Fusion DNA Polymerase	200 Units
S9455	One Fusion DNA Polymerase	1000 Units